CURRENT THERAPEUTIC RESEARCH
VOLUME 70, NUMBER 2, APRIL 2009

# Effects of Probiotic Supplementation on Markers of Acute Pancreatitis in Rats

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## ABSTRACT

**BACKGROUND:** Intestinal barrier disruption followed by bacterial translocation seems to play a role in secondary pancreatic infection in acute pancreatitis. The use of probiotics as a possible adjuvant strategy in the treatment of acute pancreatitis needs to be investigated.

**OBJECTIVE:** The aim of this study was to determine the effects of dietary supplementation with a prophylactically administered multispecies probiotic mixture on the markers of acute pancreatitis and on the occurrence of bacterial translocation.

**METHODS:** Thirty adult male Wistar rats were randomly assigned to 1 of 3 groups of 10 rats each: (1) the PS group, in which the rats were given probiotic supplementation prior to induction of acute pancreatitis; (2) the WP group, in which the rats underwent surgery to induce acute pancreatitis without prior probiotic supplementation; and (3) the control group, in which the rats underwent sham surgery. For 14 days before surgery, animals in the PS group received a single daily dose containing ~$1.2 \times 10^9$ colony-forming units of a probiotic mixture administered intragastrically as a bolus. On day 15, the animals underwent surgery to induce acute pancreatitis (PS and WP groups) or simulated surgery (control group). Blood samples were collected to determine leukocyte count, amylase and lipase activities, and glucose and calcium concentrations immediately before and 6 and 12 hours after the beginning of the procedure. Samples of pancreas, spleen, liver, and mesenteric lymph nodes were harvested for microbiologic and histopathologic analysis after the last blood sample collection. The pathologist examining the histopathology was blinded to treatment assignment.

*Accepted for publication December 22, 2008.*
doi:10.1016/j.curtheres.2009.04.004

0011-393X/$ - see front matter

**Results:** The mean leukocyte count was significantly increased in the PS group compared with the WP group ($P$ = 0.018), whereas the serum amylase and lipase activities and the serum glucose and calcium concentrations were not significantly different between the 2 groups. Comparing the risk for tissue colonization in the PS group with that of the WP group, the odds ratio (OR) for pancreas was 2.91 (95% CI, 0.13–67.10); liver, 66.55 (95% CI, 1.89–2282.66); spleen, 88.58 (95% CI, 3.04–2583.08); and mesenteric lymph nodes, 1.23 (95% CI, 0.06–25.48). When the risks for histopathologic changes were compared between the 2 groups, the OR for acinar necrosis was 1.73 (95% CI, 0.21–12.17); steatonecrosis, 12.08 (95% CI, 1.26–115.54); hemorrhage, 1.38 (95% CI, 0.21–9.53); and leukocyte infiltration, 5.91 (95% CI, 0.64–54.89).

**Conclusion:** Probiotic supplementation before the induction of acute pancreatitis was associated with a greater degree of bacterial translocation and pancreatic tissue damage in this animal model. (*Curr Ther Res Clin Exp.* 2009;70:136–148)

**Key words:** probiotics, acute pancreatitis, bacterial translocation, infection.

## INTRODUCTION

Acute pancreatitis has a wide spectrum of severity and complications. Morbidity and mortality associated with acute pancreatitis are mainly due to multiple organ dysfunction that may occur in the severe form of the disease.[1–3] Despite the large body of knowledge about the pancreas and associated diseases, treatment of acute pancreatitis continues to be a challenge.[4–6]

Pancreatic necrosis, considered the most severe local complication of acute pancreatitis, has been associated with an increased risk for mortality.[7–10] The possibility of an association between bacterial translocation and pancreatic necrosis in acute pancreatitis is being increasingly studied because the microorganisms associated with such secondary infection are gram-negative bacteria, such as those that colonize the gastrointestinal tract.[11–16]

Although some authors[17–20] have defended the use of antibiotic therapy in pancreatic infection, there is no consensus regarding its prophylactic use, even for the purpose of achieving selective decontamination of the bowel, because destabilization of the intestinal flora, alteration of the epithelial surface, and bacterial resistance may occur. A meta-analysis of 10 randomized controlled trials (RCTs) reported that the prophylactic use of carbapenem versus other antibiotics in 1279 patients with acute necrotizing pancreatitis was not associated with preventive effects on pancreatic necrosis, reduced mortality rate, or efficacy in this clinical condition.[21]

Probiotics such as *Lactobacillus plantarum* 299 have been found to have advantages over antibiotics in the prevention of bacterial translocation.[22] Probiotics produce antimicrobial factors and compete with pathogens for essential nutrients, preventing excessive pathogen growth without causing bacterial resistance.[23] By mechanisms not yet completely understood, probiotics also seem to increase the production of secretory immunoglobulin (Ig) A and prevent the proinflammatory activation of nuclear factor–κB, thereby modulating the inflammatory response.[24–26] For example, Perdigon

et al[27] found that *Lactobacillus casei*, *Lactobacillus acidophilus*, and yogurt enhanced the number of IgA-producing plasma cells in a dose-dependent manner.

Nonetheless, despite the encouraging results from studies of acute diarrhea treatment, the effectiveness of probiotics remains dubious due to the discrepancy between the results obtained in humans and animals.[28–32] Two meta-analyses of RCTs support the observation that probiotics, essentially *Lactobacillus* GG, significantly shorten the duration of acute infectious diarrhea by a mean of 18 hours (8 RCTs, 773 patients)[28] and 17 hours (7 RCTs, 675 patients).[29] In a randomized, double-blind, placebo-controlled study in 30 patients with Crohn's disease, Chermesh et al[30] found no significant differences in prevention of postsurgical recurrence of Crohn's disease between a probiotic treatment that contained a mixture of prebiotics and probiotics or placebo. Similarly, in an RCT in 129 patients scheduled to undergo major elective abdominal surgery,[31] the rate of bacterial translocation, rate of gastric colonization, and incidence of postoperative septic morbidity were not significantly changed with the administration of *L plantarum* 299v. Some studies in animals had favorable findings with probiotics in preventing microbial translocation in experimental pancreatitis.[28,32] However, the authors of a multicenter RCT trial in 298 patients with acute pancreatitis did not recommend prophylaxis with a combination of 6 strains of freeze-dried, viable bacteria in a total daily dose of $10^{10}$ bacteria, plus cornstarch and maltodextrins.[33] This controversy motivated the present study, in which dietary supplementation with a multispecies probiotic mixture in healthy animals was started before the experimental induction of acute pancreatitis to assess the effects of manipulating intestinal flora on the markers of acute pancreatitis and the preventive effects of bacterial translocation.

## MATERIALS AND METHODS

This experimental study was approved by the Ethics Committee on Animal Research at the Biology Institute Roberto Alcântara Gomes, Rio de Janeiro State University, Rio de Janeiro, Brazil. All procedures followed the current guidelines for animal experimentation.[34,35]

### Study Design

Thirty adult male Wistar rats weighing 300 to 350 g were randomly assigned to 1 of 3 groups of 10 rats each: (1) the PS group, in which the rats were given probiotic supplementation prior to surgery to induce acute pancreatitis; (2) the WP group, in which the rats underwent surgery to induce acute pancreatitis without prior probiotic supplementation; and (3) the control group, in which the rats underwent sham surgery. The animals were maintained at 23°C on a 12-hour light–dark cycle and allowed free access to water and standard laboratory chow for 14 days. Each day, the animals were weighed and their intake of water and chow was checked. During the 12 hours before the surgery, the animals were deprived of food but allowed access to water.

### Probiotics

For 14 days before surgery, the PS group received a single daily dose of ~$1.2 \times 10^9$ colony-forming units (CFU) of a multispecies probiotic mixture consisting of

3 lactobacilli (*Lactobacillus rhamnosus, L casei,* and *L acidophilus*) and 1 bifidobacterium (*Bifidobacterium longum*). Doses were prepared in an aqueous solution (5 mL) and administered intragastrically as a bolus with an orogastric catheter. Animals in the WP and control groups received 5 mL of water similarly administered once a day for 14 days before surgery.

### Surgical Procedures and Induction of Acute Pancreatitis

Acute pancreatitis was induced using the method described by Aho et al.[36] On day 15 of the experiment, after all of the animals underwent induction of general anesthesia with inhalational halothane, the PS and WP groups underwent surgery to induce acute pancreatitis by inoculation of a 5% sodium taurocholate solution into the biliopancreatic duct (1 µL/kg body weight), and the control group underwent sham surgery. After cannulation of the duct, sodium taurocholate was administered by retrograde injection at a rate of 1 µL/min under low and constant manual pressure. During the closure of the abdominal wall, 3 mL of 0.9% saline solution per 100 g of body weight was administered subcutaneously for fluid replacement. After recovery from anesthesia, the rats were allowed free access only to water.

### Collection of Blood Samples

With rats under general anesthesia induced using inhalational halothane, blood samples were harvested for laboratory analysis using cardiac puncture immediately before (0 hour) and 6 and 12 hours after the beginning of the surgery. Laboratory analysis included determination of leukocyte count (laser/impedance measurement, Automated Hematological Counter KX-21N, Sysmex Corporation, Kobe, Japan) and concentrations of amylase (direct substrate, spectrophotometry, Biosystems, Inc., Foster City, California), lipase (6-metilresorufina, Roche Diagnostic Systems, Inc., Somerville, New Jersey), glucose (glucose oxidase/peroxidase, spectrophotometry, BioSystems), and calcium (spectrophotometry, Arzenazo III, BioSystems).

### Microbiological Analysis

Twelve hours after surgery, mesenteric lymph nodes and fragments of pancreas, spleen, and liver were harvested under strict aseptic conditions and were processed for culture of aerobic microorganisms. At the end of the procedure, the animals were euthanized using an anesthetic overdose.

The samples were first cultured in brain–heart infusion broth (BHI, Oxoid Ltd., Basingstoke, United Kingdom) for 24 hours at 36°C. The samples in which bacterial growth occurred were cultured on blood agar (Oxoid Ltd.) for an additional 24 hours at 37°C. The Gram method was used to separate the microorganisms into distinct groups. Catalysis (using hydrogen peroxide) and coagulase (using rabbit's coagulase plasma) tests were applied to the samples with gram-positive microorganisms for identification of *Staphylococcus* and *Streptococcus* strains. The following tests were applied to the samples with gram-negative microorganisms: Moeller decarboxylase broth with arginine (Becton, Dickinson SA, Le Pont de Claix, France) and OF glucose (Hugh-Leifson, Merck KGaA, Darmstadt, Germany) for fermentable and nonferment-

able bacteria, respectively; and sulfide-indole-motility (Merck KGaA), double sugar (glucose and lactose) + urea,[37] and Citrate Seg Simmons Agar (Merck, São Paulo, Brazil) for enterobacteriaceae.

### HISTOPATHOLOGIC ANALYSIS

After being harvested, samples of the pancreas were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 3 to 4 μm, and stained with hematoxylin and eosin. A pathologist who was blinded to the treatment assignments assessed the slides under a light microscope. To determine the degree of acute pancreatitis, pancreatic damage was assessed using a 4-point scale that was based on the histologic features of acute pancreatitis (0 = no injury; 1 = mild injury; 2 = moderate injury; and 3 = severe injury). The features assessed were the presence or absence of acinar necrosis, steatonecrosis, hemorrhage, and leukocyte infiltration.

### STATISTICAL ANALYSIS

Block randomization was used to assign the animals to the 3 groups. The nonparametric Kruskal-Wallis test (simultaneous comparison of the 3 groups) was used to test the null hypothesis of group similarity in relation to the conditions preceding the experiment (body weight and water and food intake). A multiple linear regression model for repeated measures was used to assess the magnitude of the effect of supplementation with probiotics on the biochemical markers and leukocyte count immediately before (0 hour) and 6 and 12 hours after surgery. Multiple logistic regression analysis for repeated measurements was used to assess the independent contribution of probiotic use to bacterial growth in the pancreas, liver, spleen, and mesenteric lymph nodes, as well as the degree of pancreatic injury (acinar necrosis, steatonecrosis, hemorrhage, and leukocyte infiltration). $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using Stata version 8.0 (StataCorp LP, College Station, Texas).

## RESULTS

During the 14 days before the induction of acute pancreatitis, the groups did not behave uniformly regarding water or food consumption despite being housed in identical conditions. A significant difference between PS and WP groups was found in mean water intake ($P = 0.010$).

After the collection of the first blood sample on the day of acute pancreatitis induction, 2 animals died (1 each in the PS and control groups). Necropsy revealed blood clots in the pericardial sac, suggesting the occurrence of cardiac tamponade, possibly due to the cardiac puncture performed for blood collection. These 2 animals were excluded from the statistical analyses. All of the other animals recovered adequately from the surgical procedures and survived until the end of the experiment.

Six hours after the induction of acute pancreatitis the mean number of leukocytes was significantly increased in all 3 groups, with no significant reduction occurring at 12 hours (**Table I**). The mean leukocyte count was significantly increased in the PS group compared with the WP group ($P = 0.018$). Starting at 6 hours after study drug administration, the mean serum activities of amylase and lipase were significantly

**Table I. Effects of probiotic supplementation on leukocyte count, serum amylase and lipase activities, serum glucose and calcium concentrations, and histopathologic score 12 hours after the induction of acute pancreatitis in a rat model.**

| Laboratory Finding | PS (n = 9) vs WP (n = 10) | *P* |
|---|---|---|
| Leukocyte count, $10^3$ cells/$mm^3$ | 2.58 | 0.018 |
| Serum amylase, U/L | –338.87 | 0.234 |
| Serum lipase, U/L | 11.76 | 0.805 |
| Serum glucose, mg/dL | 6.92 | 0.497 |
| Serum calcium, mg/dL | –0.04 | 0.801 |
| Histopathologic score* | 1.77 | 0.040 |

PS = probiotic supplementation; WP = without probiotic supplementation.
*Scale: 0 = no injury; 1 = mild injury; 2 = moderate injury; and 3 = severe injury.

increased—to at least 3-fold that in animals without pancreatitis—in the PS and WP groups. The differences in serum amylase and lipase were not significant between PS and WP groups. Mean change in glycosylated hemoglobin concentrations were not significantly different between the PS and WP groups. Serum calcium concentrations were decreased progressively from baseline at 6 and 12 hours, but the between-group differences were not significant.

No growth of aerobic bacteria was observed in any of the tissue samples from the control group. Twelve hours after the start of surgery to induce acute pancreatitis in the PS and WP groups, infection was found in the pancreas (5/9 vs 3/10 rats, respectively), liver (6/9 vs 3/10 rats), spleen (4/9 vs 1/10 rats), and mesenteric lymph nodes (1/9 vs 1/10 rats) (**Table II**). On analysis of the risk for colonization of the tissues, probiotic supplementation did not protect the animals against greater colonization, especially in the pancreas (odds ratio [OR], 2.91; 95% CI, 0.13–67.10), liver (OR, 66.55; 95% CI, 1.89–2282.66), and spleen (OR, 88.58; 95% CI, 3.04–2583.08) (**Table II**).

Intestinal bacteria were identified in the organ cultures in the PS and WP groups. Gram-negative bacteria (*Escherichia coli*, 7/36 vs 3/40 organ cultures, respectively; *Pseudomonas aeruginosa*, 11/36 vs 4/40 organ cultures) and gram-positive bacteria (*Staphylococcus aureus*, 0/36 vs 1/40 organ cultures) were identified.

On histopathologic examination of the pancreas, the following degenerative injuries were found in the PS and WP groups: acinar necrosis (median scores, 2.0 vs 1.8 points, respectively; OR, 1.73; 95% CI, 0.21–12.17), steatonecrosis (1.6 vs 0.9 points; OR, 12.08; 95% CI, 1.26–115.54), hemorrhage (1.0 vs 0.8 points; OR, 1.38; 95% CI, 0.21–9.53), and leukocyte infiltration (2.0 vs 1.6 points; OR, 5.91; 95% CI, 0.64–54.89) (**Table III**). No histologic changes were observed in the control animals.

A single histopathologic score was calculated by averaging the changes detected in the pancreatic tissue in the PS and WP groups (**Table I**). The use of probiotics

**Table II. Intestinal bacterial colonization in extraintestinal tissues 12 hours after the induction of acute pancreatitis in a rat model.**

| Tissue Colonization | Number of Positive Cultures PS (n = 9) | WP (n = 10) | Control (n = 9) | Odds Ratio* PS vs WP | 95% CI |
|---|---|---|---|---|---|
| Pancreas | 5/9 | 3/10 | 0/9 | 2.91 | 0.13–67.10 |
| Liver | 6/9 | 3/10 | 0/9 | 66.55 | 1.89–2282.66 |
| Spleen | 4/9 | 1/10 | 0/9 | 88.58 | 3.04–2583.08 |
| Mesenteric lymph nodes | 1/9 | 1/10 | 0/9 | 1.23 | 0.06–25.48 |

PS = probiotic supplementation; WP = without probiotic supplementation.
*Multiple logistic regression analysis for repeated measurements was used to assess the independent contribution of probiotic use to bacterial growth in the pancreas, liver, spleen, and mesenteric lymph nodes.

was not found to protect the animals from more severe injury: the difference in this score between the PS and WP groups was significant ($P = 0.040$). The risk for steatonecrotic histopathologic damage was greater in the PS group (OR = 12.08; 95% CI, 1.26–115.54).

## DISCUSSION

Probiotics are live microorganisms that, administered in sufficient amounts, have been associated with beneficial effects.[38] Numerous studies have contributed to the understanding of probiotics.[22,32,39,40] However, more information is needed regarding their effects in various clinical conditions.[23]

In the present study, supplementation with a multispecies probiotic mixture before the induction of acute pancreatitis in rats was not found to be beneficial. Inadequate

**Table III. Histopathologic changes after the induction of acute pancreatitis in a rat model.**

| Histopathologic Change | Median Score* PS (n = 9) | WP (n = 10) | Odds Ratio† PS vs WP | 95% CI |
|---|---|---|---|---|
| Acinar necrosis | 2.0 | 1.8 | 1.73 | 0.21–12.17 |
| Steatonecrosis | 1.6 | 0.9 | 12.08 | 1.26–115.54 |
| Hemorrhage | 1.0 | 0.8 | 1.38 | 0.21–9.53 |
| Leukocyte infiltration | 2.0 | 1.6 | 5.91 | 0.64–54.89 |

PS = probiotic supplementation; WP = without probiotic supplementation.
*Scale: 0 = no injury; 1 = mild injury; 2 = moderate injury; and 3 = severe injury.
† Multiple logistic regression analysis for repeated measurements was used to assess the independent contribution of probiotic use to the degree of pancreatic injury.

doses or strains of probiotic bacteria or the time of administration may account for these findings. However, our findings did support those from several previously published clinical studies[30,31,41] and an experimental model.[42] A prospective RCT by Woodcock et al[41] found that the increase in IgA observed in the intestinal mucosa in response to probiotics in animal studies did not occur in humans after *L plantarum* 299v supplementation. Bauer et al[42] found that *Lactobacillus* GG ($1–2 \times 10^9$ CFU/d for 8–10 days) did not prevent bacterial translocation and ascitic fluid infection in rats despite successful intestinal colonization.

Although probiotics are considered to be well tolerated,[38] these live microorganisms may be associated with adverse events (eg, infection, deleterious metabolic activity, excessive immunologic stimulation, gene transfer to the endogenous flora) in susceptible individuals.[23] Thus, this study used daily probiotic concentrations not exceeding $10^9$ CFU per animal. This choice was supported by the significantly greater elevation in leukocyte count in the PS group compared with the WP group.

Results from similar studies have been controversial.[22,39] Van Minnen et al[39] used a mixture of probiotics (*L acidophilus, L casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum,* and *Bifidobacterium infantis*) at a daily dosage of $5.0 \times 10^9$ CFU/mL and observed a significant reduction in the supergrowth of pathogens (eg, *E coli*) in the duodenum; a reduction in bacterial translocation to distant organs, including the pancreas; improvement in the clinical course; and a reduction in late mortality. Mangiante et al[22] obtained analogous findings in the occurrence of bacterial translocation after supplementation with *L plantarum* 299v when using a higher concentration of probiotics (5 mL/d of a suspension containing $0.5–1.0 \times 10^9$ CFU/mL). Nonetheless, PROPATRIA (Probiotic prophylaxis in predicted severe acute pancreatitis),[33] an RCT in 298 patients with a diagnosis of severe acute pancreatitis, found that compared with placebo, prophylaxis with a combination of probiotics (*L acidophilus, L casei, L salivarius, L lactis, B bifidum*, and *Bifidobacterium lactis*) was not associated with reductions in the risk for infectious complications (OR = 1.06; 95% CI, 0.75–1.51) or in mortality (OR = 2.53; 95% CI, 1.22–5.25). A possible explanation for this negative result is that probiotic bacteria may have enhanced oxygen demand that was not supplied completely due to the presence of intestinal ischemia, which is common in such patients. Alternatively, the inflammatory response of the mucosa may have been caused by the presence of the probiotic bacteria themselves because gut epithelial cells under metabolic stress seem to react to commensal bacteria with an inflammatory response.[43]

Our negative findings might have been due to the strains of probiotic bacteria used. The strains were chosen based on studies of the effects of various probiotics on immune response and their ability to survive passage through the digestive tract.[44–48] We used a multispecies probiotic mixture because several studies recommended preparations with multiple strains, as no single strain possesses all of the desired properties.[49,50] Some authors have reported that *L rhamnosus*, one of the strains used in our multispecies mixture, was isolated from 11 patients (12%) with *Lactobacillus*-induced bacteremia.[51,52] However, *L rhamnosus* used in multispecies probiotic mixtures may be advantageous because *Lactobacillus*-induced bacteremia is a rare phenomenon that

mainly occurs when *Lactobacillus* is administered separately, especially in immunosuppressed patients.[53]

The present study had additional limitations. The probiotics were administered prophylactically for 14 days prior to the induction of acute pancreatitis. Although acute pancreatitis cannot be anticipated, it is a complication in 1% to 40% of patients who undergo endoscopic retrograde cholangiopancreatography (ERCP).[54–56] Although preventing post-ERCP pancreatitis was not the aim of the present study, the prophylactic administration of probiotic may be of value in such cases.

The ideal time for the institution of a prophylactic measure in acute pancreatitis has also been the subject of investigation. Two randomized, double-blind studies and one experimental study found that the initiation of probiotics or symbiotics after the onset of acute pancreatitis was effective.[57,58] In the first clinical study, significant reductions in the incidences of infectious complications and hospitalizations were found with *L plantarum* 299 used in 45 patients with pancreatitis.[57] The same result, in the second clinical study, was found with the use of "Synbiotic 2000" (4 different lactobacilli preparations with $10^{10}$ CFU, and prebiotics containing 4 bioactive fibers) in 62 patients.[58] The use of *Streptococcus thermophilus*, *L acidophilus* probiotics, and *B lactis* was found to significantly reduce the severity of acute pancreatitis in an experimental study.[40] In 2 experimental studies in Lewis rats, probiotic supplementation with *L plantarum* (5 mL with $0.5 \times 10^9$ cells/mL during 4 days before and 4 days after the induction of pancreatitis),[22,32] the authors recommended that supplementation be started as early as possible and continued after the onset of the signs and symptoms of acute pancreatitis.

Because bacterial translocation, which is potentially the cause of pancreatic infection and necrosis, often occurs in the early phases of acute pancreatitis,[59] blood and tissue samples were collected within 12 hours after the induction of pancreatitis. The high rates of infection of extraintestinal organs, including the pancreas, found in the present study support the findings from previously published studies,[60,61] suggesting that *E coli*, *Fusobacterium* spp, *Proteus mirabilis*, or *Propionibacterium* spp are the bacteria usually responsible for the contamination of pancreatic necrosis.[60]

The microbiologic study of pancreatic sepsis in humans is well characterized and reflects common gastrointestinal flora.[62] The frequently isolated microorganisms include *E coli, Pseudomonas* spp, *Proteus* spp, *Acinetobacter* spp, *Streptococcus* spp, *Staphylococcus* spp, and *Candida* spp, among others. The strains of bacteria isolated in pancreatic necrosis may vary depending on the etiology of the acute pancreatitis. Gram-negative bacteria occur frequently in biliary pancreatitis, whereas gram-positive organisms are more common in alcoholic pancreatitis.[63] Our findings were similar to those in infected pancreatic necrosis in humans, but we did not test for anaerobic bacteria or fungi.

Several clinical studies have found that the prophylactic use of probiotics did not appear to be a promising alternative to the use of antibiotics.[31,41,64] Two randomized clinical studies assessed the effects of the use of probiotics and symbiotics on the incidences of bacterial translocation, gastric colonization, and septic complications in patients scheduled to undergo elective surgery.[31,64] McNaught et al[31] used *L plantarum* and Anderson et al[64] used a symbiotic (oligofructose and a multispecies mixture of

probiotics) in 129 and 144 patients, respectively; both studies found that this prophylactic use had no significant clinical benefit. Woodcock et al[41] investigated the role of *L plantarum* in the function of gut-associated lymphoid tissue in 22 patients scheduled for elective surgery and found no significant increases in IgA or IgM concentrations in the intestinal mucosa, a finding that differs from usual findings in animals.[65,66]

Because of contradictory findings, we believe that further studies are needed using other strains of probiotic bacteria administered at various concentrations and at different times in relation to pancreatic injury to determine their role in bacterial translocation in rats.

## CONCLUSION

In the present study, probiotic supplementation before the induction of acute pancreatitis was associated with a greater degree of bacterial translocation and pancreatic tissue damage in this animal model.

## ACKNOWLEDGMENTS

This work was partially supported by a grant from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

The authors thank Yugo de Lima Brandão Mirakami, MD, Department of Pharmacology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, and Denilson Ferreira Batista, Department of Microbiology, Immunology, and Parasitology, Rio de Janeiro State University, Rio de Janeiro, Brazil, for their technical assistance.

Ms. Horst, Dr. Marques, Ms. Diestel, Ms. Matzke, Ms. Simões, and Dr. Melo participated in the study design, research, and data collection. Ms. Horst and Drs. Marques, Braga, and Portela designed and realized the statistical analysis. Drs. Andrade, Lobão, and Vaz performed the biochemical, microbiologic, and histopathologic analyses of the data. All of the authors read and approved the manuscript.

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